





Ovid: Lockyer: J Cardiovasc Pharmacol, Volume 33(5).May 1999.718-725

Page 1 of 17

EXHIBIT 10

   	Full Text
Results Display Main Search Page Ask an Information Specialist Online Support Help LOGOFF	

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Demonstration of Flow and Platelet Dependency in a Ferric Chloride-Induced Model of Thrombosis [Articles]

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Summary:

Further to characterize the processes involved in the FeCl_3 -induced thrombosis model, we determined the effect of aspirin, heparin, hirudin, *trans*-4-(aminomethyl) cyclohexane carboxylic acid (AMCHA), thrombocytopenia, and flow modifications on time to occlusion (TTO) and thrombus weight (TW) in the rat carotid artery. Aspirin, from 3 to 100 mg/kg, showed no dose-response relation for either TTO or TW and did not significantly affect *ex vivo* platelet aggregation. Heparin, at doses that significantly increased the activated partial thromboplastin time (APTT), dose-dependently increased the TTO of animals that showed an occlusion during the monitoring period and also reduced the TW. Hirudin required constant infusion to prevent occlusion and reduce the TW, when the APTT was also significantly increased. AMCHA did not affect the TW but reduced the TTO. Animals made thrombocytopenic by the use of antiplatelet serum did not occlude during the monitoring period, and the TW was significantly reduced. Changes in flow showed that the TTO was not affected, but the TW showed an inverse correlation with average flow. The results obtained for platelet depletion

and flow modifications expand on previous findings with this model and support the physiological relevance of the model.

A number of experimental models of thrombosis have been designed to facilitate rapid screening of potential antithrombotic compounds. A modification of the ferric chloride-induced model of thrombus formation in the rat carotid artery (1) was used to study the effect of antithrombotic compounds and more fully to investigate the factors involved in thrombus formation.

Models of arterial thrombosis are useful in the screening of potential antithrombotic compounds, but the success of the model in predicting clinical efficacy and in deciding the appropriate indication would be greatly improved if the mechanism(s) involved were more fully understood. The FeCl_3 -induced model of thrombosis has been used as a model of a mixed-type, occlusive arterial thrombosis, but the role of some important factors involved in the formation of the thrombus is not clearly defined. We investigated the role of platelets, the fibrinolytic system, the effect of flow modifications, and the effect of the direct thrombin inhibitor hirudin, in addition to some compounds previously tested, in this model.

The involvement of platelets in this model of thrombosis has been assumed, but not directly investigated. By inducing thrombocytopenia with antiplatelet serum, it is possible to examine directly the contribution of platelets to thrombus formation. Because endothelial injury that exposes the subendothelium will lead to platelet adherence and activation, and hence thrombin release, it is probable that platelets play a major role in the initiation of thrombus formation after FeCl_3 -induced damage. The existence of platelets within the thrombi produced in thrombosis models has been consistently noted (2), but a direct examination of their importance will clarify the extent of their involvement versus the importance of the activation of coagulation and the formation of fibrin.

Thrombus formation is a manifestation of the relative contribution of

• REFERENCES

Graphics

- Fig. 1
- Table 1
- Fig. 2
- Fig. 3
- Fig. 4
- Fig. 5
- Fig. 6

Recent History

Demonstration of Flow and...



Ovid: Lockyer: J Cardiovasc Pharmacol, Volume 33(5).May 1999.718-725

Page 3 of 17

thrombogenic and thrombolytic processes. To study the contribution of the fibrinolytic system to thrombus development, we used the plasmin inhibitor *trans*-4-(aminomethyl) cyclohexane carboxylic acid (AMCHA; tranexamic acid). If the fibrinolytic system plays a significant role in the determination of the rate or degree of thrombus formation, then this should be revealed by the effect of its inhibition on time to occlusion (TTO) and thrombus weight (TW).

Changes in blood flow also are thought to affect thrombus formation; we investigated this by modifying the flow in the carotid artery by selectively ligating the ipsilateral branches of the common carotid to reduce the flow, or by ligating the contralateral artery to increase the flow. In this study we attempted to examine the effect of changing blood flow itself without the changes in turbulence normally accompanying these types of experiments.

It was the purpose of this study to manipulate these variables and processes so that the mechanisms underlying thrombogenesis in this model may be better understood.

MATERIALS AND METHODS

Arterial thrombosis

Experiments were carried out according to the method used by Kurz et al. (1), but carotid flow was continuously monitored by using an ultrasound flow probe to determine the precise time of occlusion, rather than by detection of a temperature decrease in the artery. Male Sprague-Dawley rats (weight range, 300-400 g; Charles River, Wilmington, MA, U.S.A.) were anesthetized with ketamine/xylazine (80 and 12 mg/kg, i.m., respectively), and the femoral artery and vein cannulated for blood pressure and heart rate monitoring and drug administration. The left carotid artery was exposed through a medial ventral longitudinal incision extending from the mandible to the suprasternal notch and carefully freed from its connective tissue sheath between the base of the mandible and the internal/external carotid bifurcation by blunt dissection. A piece of Parafilm was positioned under the artery to isolate it, and ligatures were placed proximally and distally around the vessel. An ultrasonic flow probe (Transonic 1RB; Transonic, Ithaca, NY, U.S.A.) was placed around the artery proximally and flow recorded via a Transonic flow meter (T106) connected to a computer running WINDAQ data-acquisition software (DATAQ, Akron, OH, U.S.A.). A 3-mm diameter filter paper disk soaked in FeCl₃ (50% wt/vol) was applied to the ventral

surface of the artery, distal to the flow probe, after a stabilization period of 15 min. Flow and blood pressure were monitored until complete occlusion of the carotid artery was detected, as indicated by a cessation of flow, or until 60 min after the application of FeCl_3 had elapsed. The time elapsed between the application of FeCl_3 and cessation of flow was recorded as the TTO. At occlusion, or after 60-min FeCl_3 application, the proximal and distal ligatures were tightened, and the complete exposed artery segment excised. The artery was freed of any adherent tissue under a dissecting microscope, and a longitudinal incision made along its length; a gentle stream of saline was used to wash out any liquid blood. The artery and thrombus were then gently blotted dry, and the segment weighed (Sartorius, Germany). The entire thrombus was then gently scraped from the artery, and the vessel wall reweighed; the TW was obtained by subtraction.

Measurement of activated partial thromboplastin time²¹

Arterial blood was withdrawn into 3.9% wt/vol sodium citrate (9:1 vol/vol, blood/citrate) and centrifuged to obtain platelet-poor plasma, which was then used within 2 h for activated partial thromboplastin time (APTT) determination. APTT was measured by using a commercially available kit (A-1801; Sigma, St. Louis, MO, U.S.A.) and a coagulation timer (Fibrometer, Cockeysville, MD, U.S.A.).

Induction of thrombocytopenia²¹

Rabbit anti-rat platelet serum (antiplatelet serum; APS) was a kind gift of the 2nd Tokushima Institute, Otsuka Pharmaceuticals, Osaka, Japan. One-tenth milliliter APS was diluted to 1 ml with 0.9% saline and injected intraperitoneally 24 h before experimentation. Blood samples were taken before administration of the APS and at the conclusion of the experiment and platelets counted (Unopette; Becton-Dickinson, Rutherford, NJ, U.S.A.) to verify that thrombocytopenia had been induced. Animals were considered thrombocytopenic if the platelet count was reduced by >95% at the time of experimentation.

Flow modifications²¹

Carotid blood flow was reduced by ligation of internal branch of the ipsilateral carotid, distal to the point at which the flow was measured, or increased by ligation of the contralateral artery.

Platelet aggregation[±]

Animals were anesthetized with ketamine/xylazine (80 and 12 mg/kg i.m., respectively), and the femoral vein and carotid artery catheterized for drug administration and blood collection. Aspirin was infused as for thrombosis studies, and the animal exsanguinated after 30 min into 20 U/ml heparin. Blood was diluted 1:1 (vol/vol) with 0.9% (wt/vol) saline and used immediately. Platelet aggregation was determined by using a Chronolog model 592 whole blood aggregometer and model 810/810-CA Aggro/link computer interface and software (Chrono-log Corp., Havertown, PA, U.S.A.). Aggregation was induced by using collagen (385 Chrono-par). A pilot study was conducted by using a range of collagen concentrations (0.5-10 µg/ml) to determine the smallest dose required to induce ~70% aggregation; that concentration (1 µg/ml) was used for the determination of the effect of aspirin on platelet aggregation.

Drug administration[±]

Drugs were obtained from Sigma and dissolved in 0.9% saline. Aspirin, 3-100 mg/kg, was given i.v. in a total volume of 300-600 µl, 30 min before application of FeCl₃; hirudin, 150-750 U/kg/30 min or 1,000 U/kg/min, 300 µl every 30 min or 25 µl/min, starting 10 min before application of FeCl₃; *trans*-4-(aminomethyl) cyclohexane carboxylic acid (AMCHA) 200 mg/kg, 300 µl i.v., and heparin (Elkins-Sinn, Cherry Hill, NJ, U.S.A.) 100-1,000 U/kg/30 min, 300 µl every 30 min, i.v., starting 15 min before FeCl₃.

Statistics[±]

Data were analyzed by using the program SigmaStat for Windows v2.0 (SPSS, San Rafael, CA, U.S.A.). Comparisons between groups were made by using an analysis of variance (ANOVA) followed by Dunnett's test, or Dunn's test where normality test failed and the treatment group sizes were not equal. The log-rank test was used to analyze TTO data, where there were animals that did not show an occlusion within the 60-min monitoring period.

RESULTS[±]

Control conditions[±]

Initial experiments were performed by using 35% (wt/vol) FeCl₃ to induce thrombosis, but this concentration led to the production of a thrombus that

sometimes broke away from the vessel wall, leading to an ill-defined TTO. Although reformation of the thrombus was rapid, often leading to reocclusion within a few minutes of embolization, the TW under these circumstances was probably not comparable with those obtained in experiments in which no embolization occurred. In all subsequent experiments, the concentration of FeCl_3 was increased to 50%, which slightly decreased the TTO and gave a more reproducible end point by reducing the incidence of embolization.

The TTO and TW were found to be very reproducible in control experiments (Fig. 1, control), which were repeated routinely during the course of this series of investigations. TW and TTO were chosen as experimental variables to represent the thrombotic process(es) because they are unrelated variables, but both are measures of the effect of drugs and other interventions on thrombus formation. Calculation of the correlation coefficient for pairs of TW and TTO values shows that these parameters are not correlated.



FIG. 1. Effect of aspirin on time to occlusion and thrombus weight. Control (n = 12); aspirin (mg/kg), 3 (n = 9), 10 (n = 6), 30 (n = 12), 100 (n = 5). Data are expressed as mean \pm SEM. *p < 0.05 vs. control (Dunnett's test).

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Antiplatelet serum

In two animals that received APS, but which were not thrombocytopenic by our criterion (platelet reduction, 73.7 and 70.4%), there was no significant reduction in TW; however, the TTO was significantly prolonged.

Reduction of the platelet counts by >95% prevented occlusion of the carotid during 60 min of FeCl_3 application in all animals that were thrombocytopenic to this degree. TW was also significantly reduced, compared with control. Results are summarized in Table 1.

Group	Thrombus weight (mg)	Time to occlusion (min)	Thrombus weight (mg)
Control (n = 12)	7.3 ± 1.0	33.1 ± 5.1	7.2 ± 0.5
Thrombus weight (n = 12)	6.1 ± 0.2		6.2 ± 0.3

Values represent mean ± SEM.
*p < 0.05 vs. control (log rank test).
**p < 0.01 vs. control (Dunn's test).

TABLE 1. Effect of APS-induced thrombocytopenia on time to occlusion and thrombus weight

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Aspirin

Aspirin did not produce a dose-dependent effect on either TTO or TW. The average TW was decreased at all doses, but this reduction was significant at only 10 and 30 mg/kg. TTO was increased at 3 and 10 mg/kg; the effect was more pronounced at the higher dose, but none of the changes was statistically significant. The highest dose of aspirin also failed significantly to prolong the TTO or to reduce the TW. Results are presented in Fig. 1.

Heparin

Heparin caused a dose-dependent increase in TTO, which was significant at all doses (log-rank test); no occlusions occurred at 1,000 U/kg. The APTT was increased >400 s at each of these doses. TW was dose-dependently reduced, and the reduction was statistically significant at the higher doses. Results are shown in Fig. 2. The consistency of the thrombus was different after heparin administration and was less well developed and was of a softer nature than with FeCl₃ alone. This made the thrombus somewhat difficult to weigh because blotting of the saline used to rinse the thrombus carried with it the risk of removing some of the thrombus itself. Therefore blotting was carried out much more gently, and for this reason, the TW may be slightly overestimated in the heparin experiments.



FIG. 2. Effect of heparin on time to occlusion and thrombus weight. Control (n = 12); heparin (U/kg/30 min), 100 (n = 4), 300 (n = 6), 1,000 (n = 4). Data are expressed as mean ± SEM. *p < 0.005 vs. control (log rank test). **p < 0.05 vs. control (Dunn's test).

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Hirudin

Hirudin was ineffective in prolonging the TTO when administered every 30 min, when the APTT was increased approximately twofold over control. However, a constant infusion of hirudin completely prevented occlusion; the APTT was increased threefold over control by 10-min infusion and increased >600 s thereafter. Hirudin also was ineffective in reducing TW after 30-min dosing, but continuous infusion produced a statistically significant reduction. Results are presented in Fig. 3.



FIG. 3. Effect of hirudin on time to occlusion and thrombus weight. Control (n = 12); heparin (U/kg/30 min), 150 (n = 4), 300 (n = 3), 700 (n = 4), infusion (n = 3). Data are expressed as mean \pm SEM. *p < 0.05 vs. control (Dunnett's test).

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AMCHA

AMCHA did not significantly affect TW, but significantly reduced the TTO (see Fig. 4). The dose of AMCHA used in this study was previously shown to inhibit fibrinolysis (3). Attempts to monitor the change in serum fibrin(ogen) degradation products (FDPs) after AMCHA administration, by using a monoclonal antibody-based latex agglutination assay, were unsuccessful. The test kit relies on a human monoclonal antibody to recognize fibrinogen, which did not cross-react with rat fibrinogen.

FIG. 4. Effect of AMCHA on time to occlusion and thrombus weight. Control (n = 12), AMCHA (n = 8).



Data are expressed as mean \pm SEM. * $p < 0.05$ vs. control (Dunnett's test).

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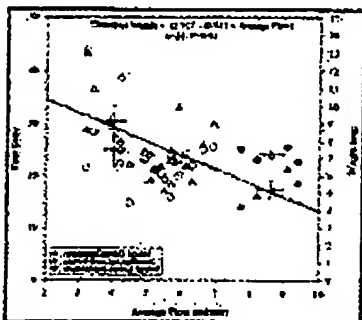
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The consistency of the thrombus appeared more solid after AMCHA treatment. On removal of the thrombus for weighing, it became detached in a single solid piece, rather than as softer particles, as under control conditions.

Flow modifications

Flow reduction in the common carotid was achieved by ligation of the internal or external branch. Flow was quantified as the change in average flow measured over the duration of the period between the application of FeCl_3 to the point at which the flow was obviously affected by thrombus formation. It was found that ligation of the internal carotid produced a more prolonged decrease in flow than did ligation of the external branch; ligation of the former produced an $\sim 18\%$ reduction in flow, but the flow returned to baseline during the course of the experiment, presumably through a compensatory mechanism. Alterations in blood flow were inversely correlated with a change in TW (Fig. 5; $p < 0.01$), but did not affect the TTO. An increase in average flow caused a statistically significant reduction in TW, whereas a decrease brought about an increase, which also approached significance. It was observed that the thrombus formed under low-flow conditions was noticeably longer than that of the control and often extended from the point of application of FeCl_3 to the bifurcation of the artery. Carotid ligations did not affect systemic blood pressure or heart rate.

FIG. 5. Correlation of time to occlusion (TTO) and thrombus weight with average flow. Control ($n = 11$), reduced flow ($n = 8$), increased flow ($n = 4$); circles, TTO; triangles, thrombus weight. Error bars are mean \pm SEM.



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Platelet aggregation

A preliminary in vitro study was conducted to investigate the effect of aspirin on platelet aggregation in whole blood. Aspirin was found to inhibit aggregation dose dependently at relevant concentrations (0.1 and 1.0 mM, final concentration; data not shown). In the ex vivo study, platelet aggregation was increased at 3 mg/kg and decreased at higher doses, compared with control, although these differences were not statistically significant. Data are presented in Fig. 6.

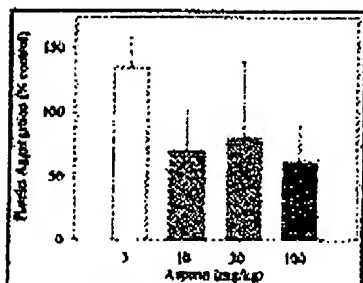


FIG. 6. Effect of aspirin on ex vivo platelet aggregation. Platelet aggregation was induced by using 1 μ g/ml collagen control (n = 3); aspirin (mg/kg) 3 (n = 3), 10 (n = 3), 30 (n = 3), 100 (n = 3). Data are expressed as mean \pm SEM.

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DISCUSSION

The experimental models developed for the induction and study of thrombus

formation in the large vessels of experimental animals *in vivo* use a variety of stimuli to promote thrombosis; these include crush injury followed by stenosis (Folts model; 4), balloon catheter-induced damage (5), electrical injury (6), copper coil (7), heating (8), insertion of a silk thread (9), and chemical injury (1). These techniques rely on exposure of the thrombogenic subendothelial layer to the bloodstream, after formation of the arterial lesion. The ferric chloride model was chosen because it causes the formation of a "mixed thrombus," that is, one composed of fibrin, activated platelets, and entrapped erythrocytes. This is the type of thrombus that is found in the coronary arteries after sudden death and acute myocardial infarction (10). The model is reliable, has given reproducible results, and has the added advantage of being simple and quick to perform. The endothelial damage induced by the FeCl_3 was shown microscopically to include separation of endothelial cell junctions and denudation (1), thus exposing the subendothelium. For these reasons, we chose this model to investigate the relative contribution of the factors involved in thrombogenesis.

Pentobarbital has often been used as an anesthetic in these types of experiment, but it was shown to decrease platelet adherence in the rat *in vivo* (11) and to alter platelet function *in vitro* by suppressing aggregation and the release reaction induced by collagen or low concentrations of thrombin (12). To study the contribution of platelets to thrombus formation, without the possible interference of pentobarbital, the combination of ketamine and xylazine was chosen. These agents, which are commonly used together to induce surgical anesthesia in rodents, are not documented to have any effect on platelet function.

The contribution of platelets in this type of thrombosis model was investigated previously by subjecting the animals to whole-body irradiation (13). However, whole-body irradiation was shown to affect the levels of thromboxane B_2 (TXB_2) and prostacyclin in rats (14) and may therefore alter the process of thrombogenesis by means unrelated to platelet depletion. By using an antiserum raised against rat platelets, it was possible to induce profound thrombocytopenia and to investigate the participation specifically of platelets in thrombus formation, without affecting other components of the hemostatic process. The prevention of thrombus formation in all animals that were thrombocytopenic by our criterion demonstrates that the involvement of platelets is obligatory in thrombogenesis in this model. This finding is in agreement with the mode of action of FeCl_3 in this model, which exposes the subendothelium to the blood-stream (*vide supra*). This exposure leads to platelet adherence and activation, providing the phospholipid

surface necessary for thrombin generation and activation of the coagulation cascade, hence leading to the formation of a mixed-type thrombus. It is apparent that the deposition of platelets on this surface is a prerequisite for thrombus development.

The effects of direct or indirect inhibition of thrombin by hirudin and heparin, respectively, allowed an examination of the involvement of coagulation. At doses that significantly increase the APTT, both modes of thrombin inhibition affect thrombus formation. The effect of heparin was dose dependent both in its prolongation of TTO and in its reduction of TW, but hirudin was effective only when given as an infusion. The results obtained with heparin are in agreement with those previously obtained in a similar model, which used FeCl_3 as the thrombogenic stimulus (15), and it was noted previously that a large increase in APTT is necessary for a heparin effect to be observed (16). This may be partially due to the neutralization of the heparin by the platelet-rich thrombus induced in this model (17).

Constant infusion of hirudin was required to induce any significant change in thrombus formation, even though the APTT was increased by the bolus administrations. Hirudin was investigated in an FeCl_3 -induced thrombosis model in the rabbit, in which the TW and TTO were affected in a dose-dependent manner (18). However, in these experiments, the concentration of FeCl_3 used was 70% (wt/vol) versus 50% (wt/vol) in our experiments. The higher concentration was found to be necessary because a lower concentration of 35% (wt/vol) was found to be unable to stimulate thrombus production, whereas that same concentration is able to induce thrombus formation in our model (data not shown). These discrepancies indicate that the thrombotic process may be different in rabbits and rats; that a stronger thrombogenic stimulus was used also may explain why thrombus formation was observed at lesser increases of APTT in the rabbit model.

The formation of a thrombus is the result of a disturbance of the normal balance between thrombogenesis and thrombolysis; if an increase in thrombogenesis outweighs the counterbalancing effect of the basal activity of the fibrinolytic system, thrombus formation occurs. As a result of this, it is perhaps not surprising that inhibition of the fibrinolytic system by AMCHA significantly reduced the TTO by allowing the thrombus to form more quickly than with FeCl_3 alone. Because TW was not reduced under these conditions, it is possible that thrombus formation proceeds faster in the area of FeCl_3 application when

fibrinolysis is inhibited and leads to occlusion before thrombus development progresses distally from the site of damage. Under control conditions, thrombus growth can progress longitudinally, which allows thrombus size and mass to increase before occlusion is achieved.

Aspirin was previously shown to be ineffective in a very similar model, which uses ferrous chloride to induce injury (19), but the highest dose investigated was 50 mg/kg. Other articles showed a variable effect of aspirin over a wide dose range (6,20), but none used the ferric chloride-induced model of thrombosis. The upper limit of the dose range was extended to check for any dose-response relation at higher concentrations. Rat tissue cyclooxygenase also is resistant to aspirin, and high doses may be required for total inhibition (21). At these higher concentrations, there was no evidence of any significant decrease in TW or increase in TTO. The lack of effectiveness of aspirin, even at doses that should be sufficient to inhibit production of TXA_2 , is possibly explained by its inhibitory effect on the production of prostacyclin. It is probable that there is a complex interrelation between the effect of aspirin on the inhibition of production of these metabolites (20). The relative degree of inhibition of the production of each metabolite will have variable effects on the tendency to thrombogenesis in the presence of a suitable stimulus. This may result in the observed lack of a dose-response relation in the effect of aspirin on TTO or TW. Additionally, aspirin may not be effective because of the low level of production of TXA_2 by rat platelets (22). In this case, inhibition of TXA_2 production may not have a noticeable effect on thrombus formation and may explain why the TW was reduced at lower doses, but was increased at the higher doses. A similar pattern of observations was reported in a rat arterial crush model (23).

Aspirin was found not to have a significant effect on aggregation in the ex vivo platelet study, although aggregation was reduced at the higher doses. This effect at doses >3 mg/kg may have contributed to the lower TWs observed at the same doses. The lack of a clear dose-related aspirin effect on TW or TTO, therefore, is probably explained by the mixed nature of the thrombus formed in this model and the complexity of factors contributing to its development and their interrelation.

Flow modifications showed that, although there was no significant effect on the TTO, there was a significant increase in TW when the average flow was decreased; a similar but an opposite effect was observed under conditions of

increased flow. One possible explanation for these findings is purely mechanical: under low-flow conditions, the platelets are less likely to be dislodged by the smaller hemodynamic forces exerted on them, leading to the aggregation of a larger number of platelets, and with them, the associated fibrin and entrapped erythrocytes, thus leading to a more massive thrombus. This suggestion is supported by the observation of increased thrombus length in the animals with ipsilateral ligations. Under conditions of increased flow, platelets that are less tightly attached to the endothelium and to each other would be more likely to be dislodged (24), leading to the development of a smaller thrombus. In addition, the endothelium is reported to be more fibrinolytic under high shear-stress conditions (25) and, even though a portion of the endothelium may be damaged by the FeCl_3 application, it is possible that enough functionally intact endothelium remains to affect the thrombus development under increased flow conditions. The findings of this series of experiments are in agreement with those obtained by using a superfused monolayer of endothelial cells (26). That the TTO remains unchanged indicates that the rate of transverse growth of the thrombus, which leads to the occlusion, probably remains unchanged, and only the longitudinal growth is affected. This could be the case because the endothelium is damaged at the site of FeCl_3 application and cannot exert any effect on the process of thrombosis in this region, but its actions proximally and distally would be unaffected. Platelets are known to be activated under conditions of increased shear rate (27), and this also contributes to increased thrombogenesis under high-flow conditions.

At the completion of each experiment, a section of the FeCl_3 -treated artery was removed for sectioning and hematoxylin and eosin staining to allow examination of the histologic composition of the thrombus. It was hoped that this would reveal differences between the groups that would allow some insight into the basis for the experimental observations. With the exception of the APS-treated group, in which no thrombus formation was observed, we did not find any such differences. Possible explanations for this include difficulty in identifying sections in different thrombi that correspond exactly to one another in terms of their physical location, and also the heterogeneity of thrombus composition, which can result in considerable differences in the histologic appearance of the sections, even though they were taken very close to one another. Both these factors complicate the task of identifying alterations in composition, which may be indicative of the physiological effect of the treatment.

In summary, the FeCl_3 -induced model of thrombosis is dependent on the interaction of platelets, thrombin, fibrinolysis, and blood flow. Manipulation of these components results in alterations of the thrombogenic process, resulting in changes in the TTO or the TW. The ability of heparin and hirudin to affect thrombus development indicates the involvement of thrombin in this model. Taken together, the results of the experiments with APS, heparin, and hirudin indicate that, in this model, an interplay between platelets and the coagulation and fibrinolytic systems occurs during thrombus formation and that the participation of platelets is of absolute importance. Each of these factors was demonstrated to be of importance in thrombogenesis, and the fact that changes in any of them bring about changes in the parameters that define the process of thrombosis indicates the physiological relevance of this model.

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Ovid: Lockyer: J Cardiovasc Pharmacol, Volume 33(5).May 1999.718-725

Page 16 of 17

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Page 17 of 17

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